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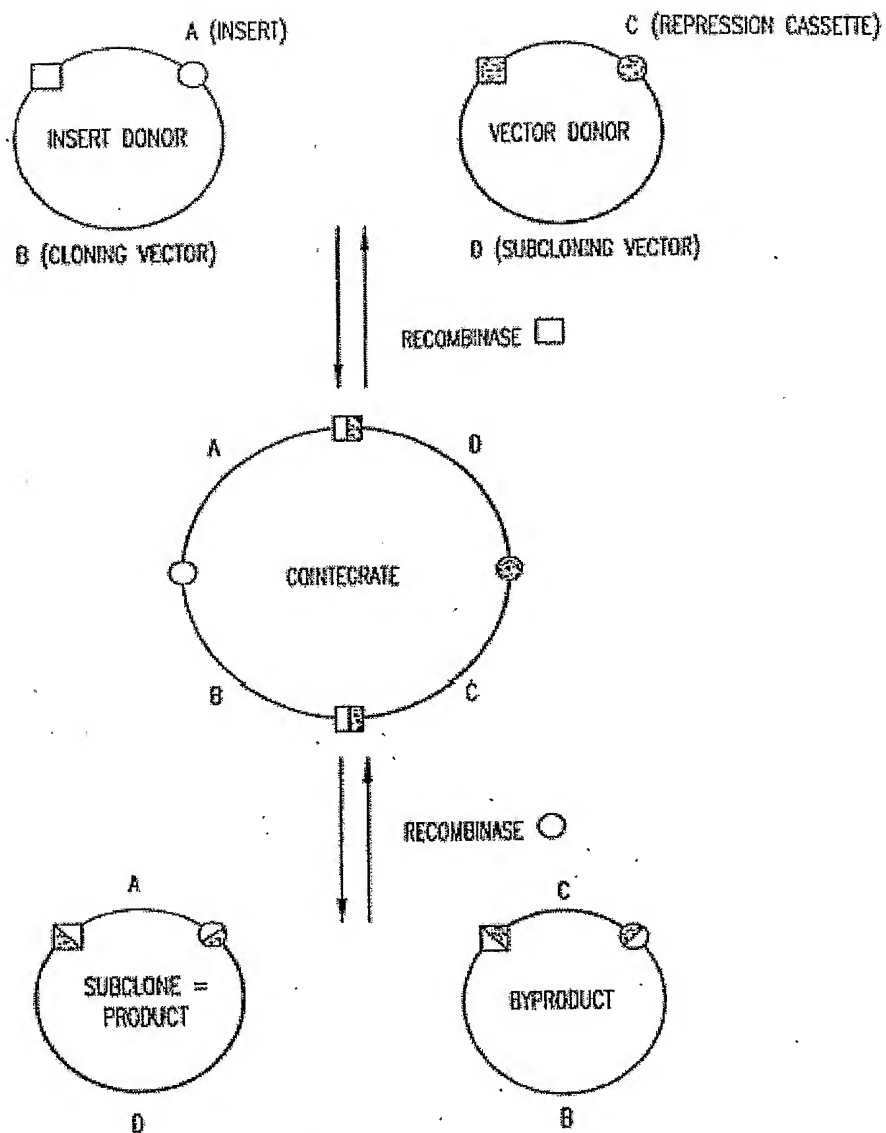


FIG.1

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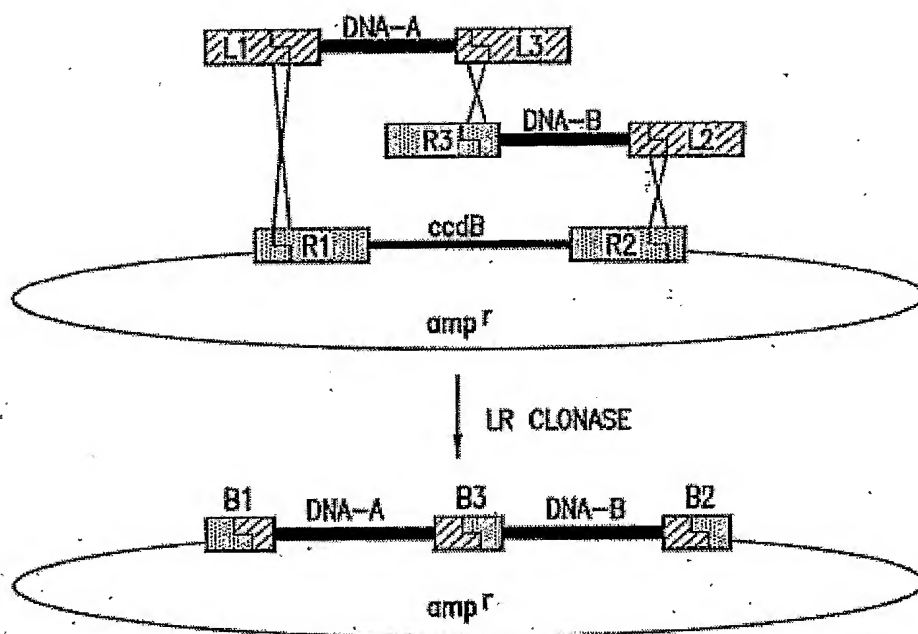


FIG.2

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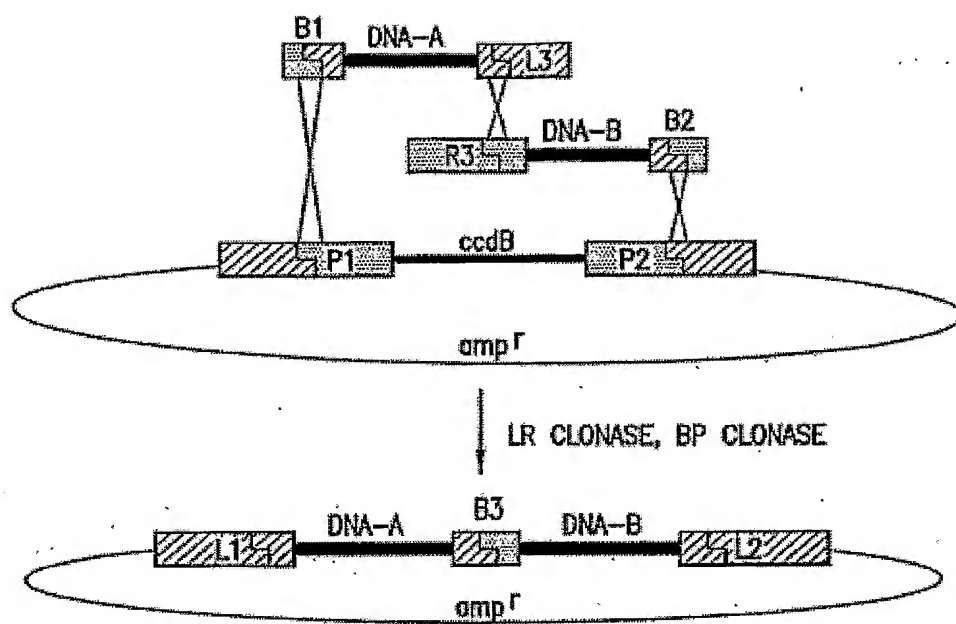


FIG.3

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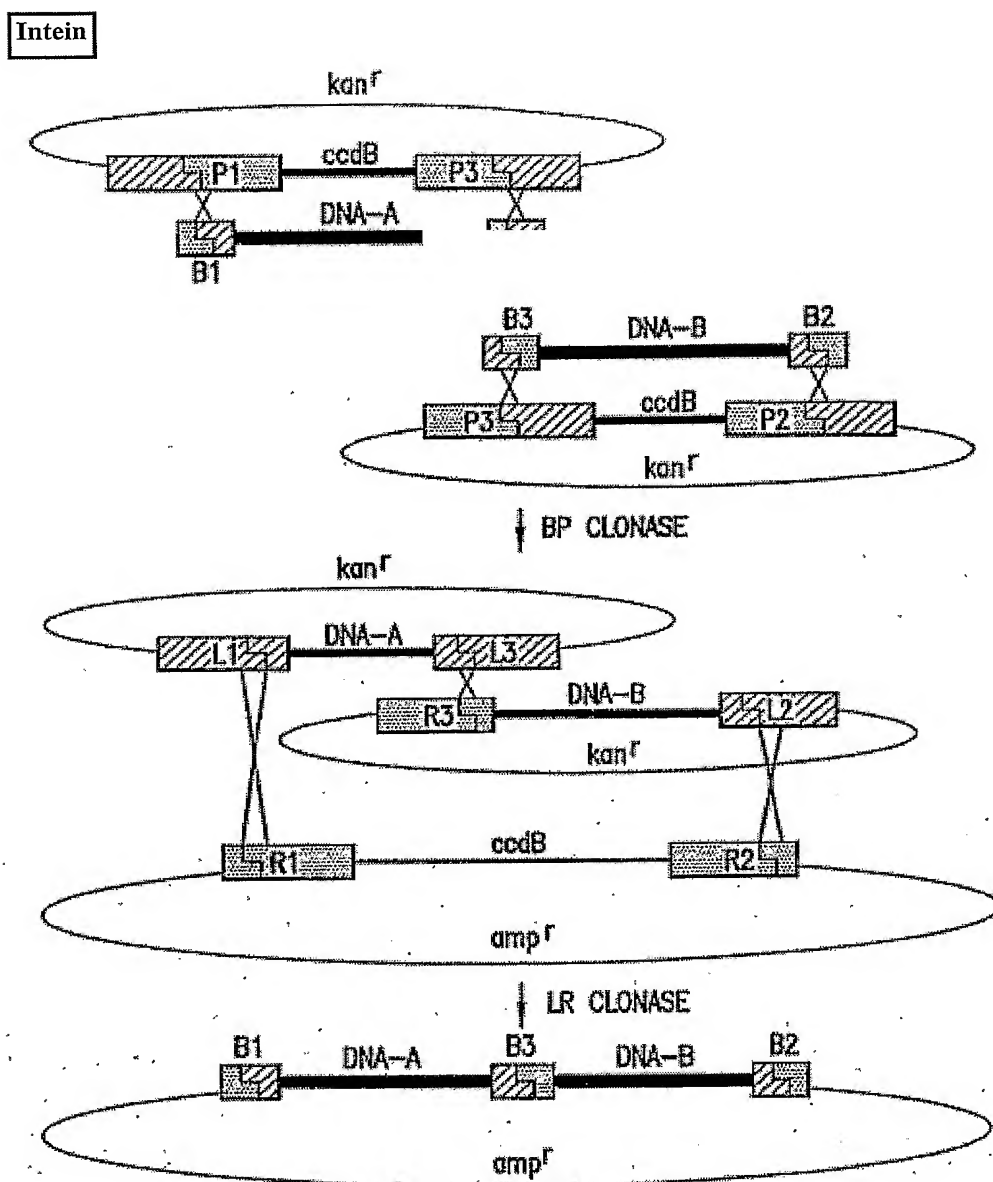
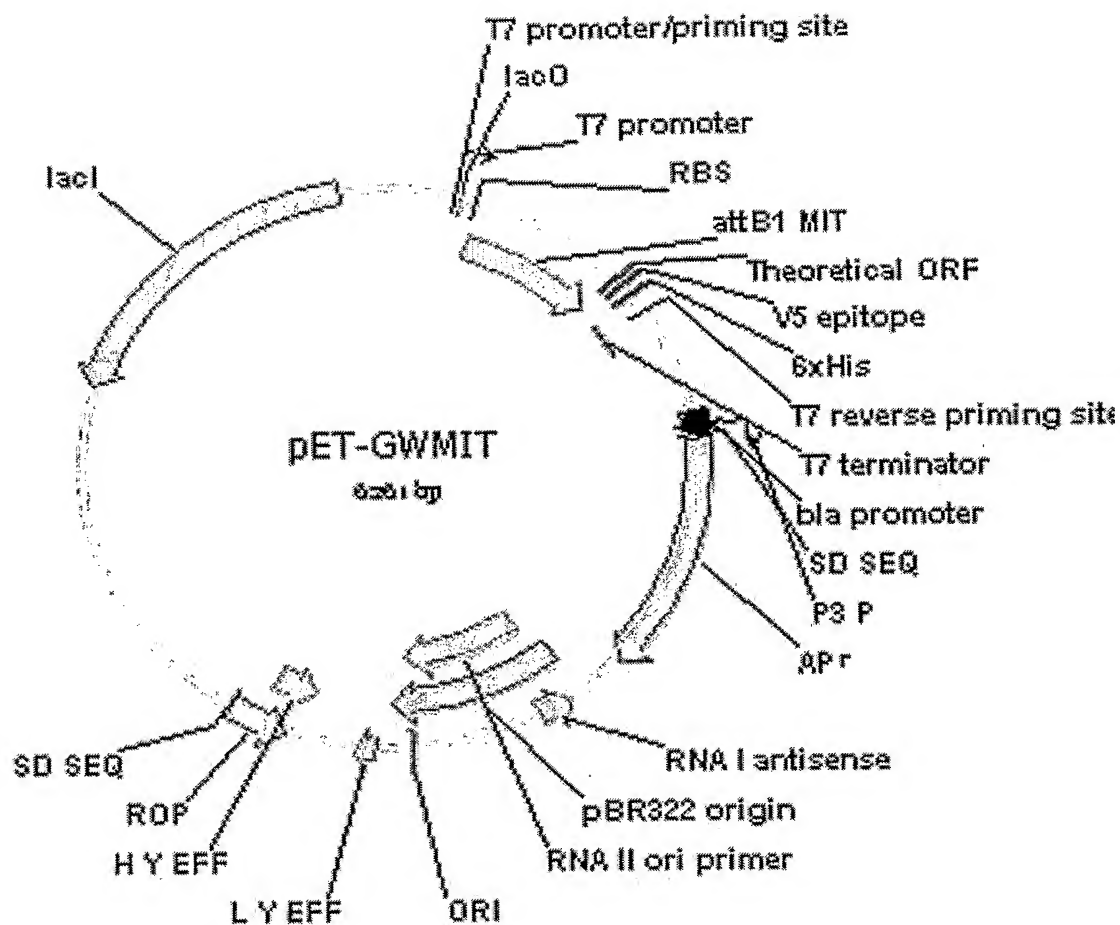


FIG.4

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**FIG. 5**

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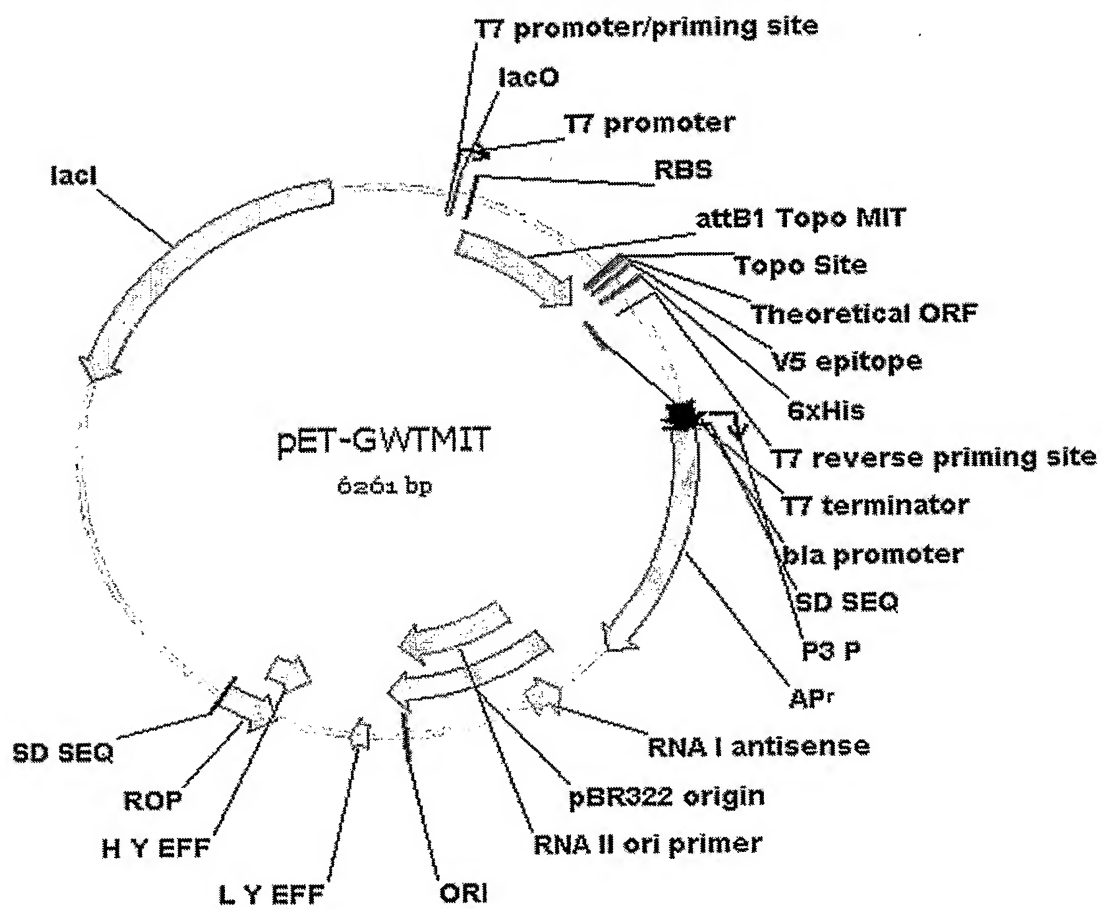


FIG. 6

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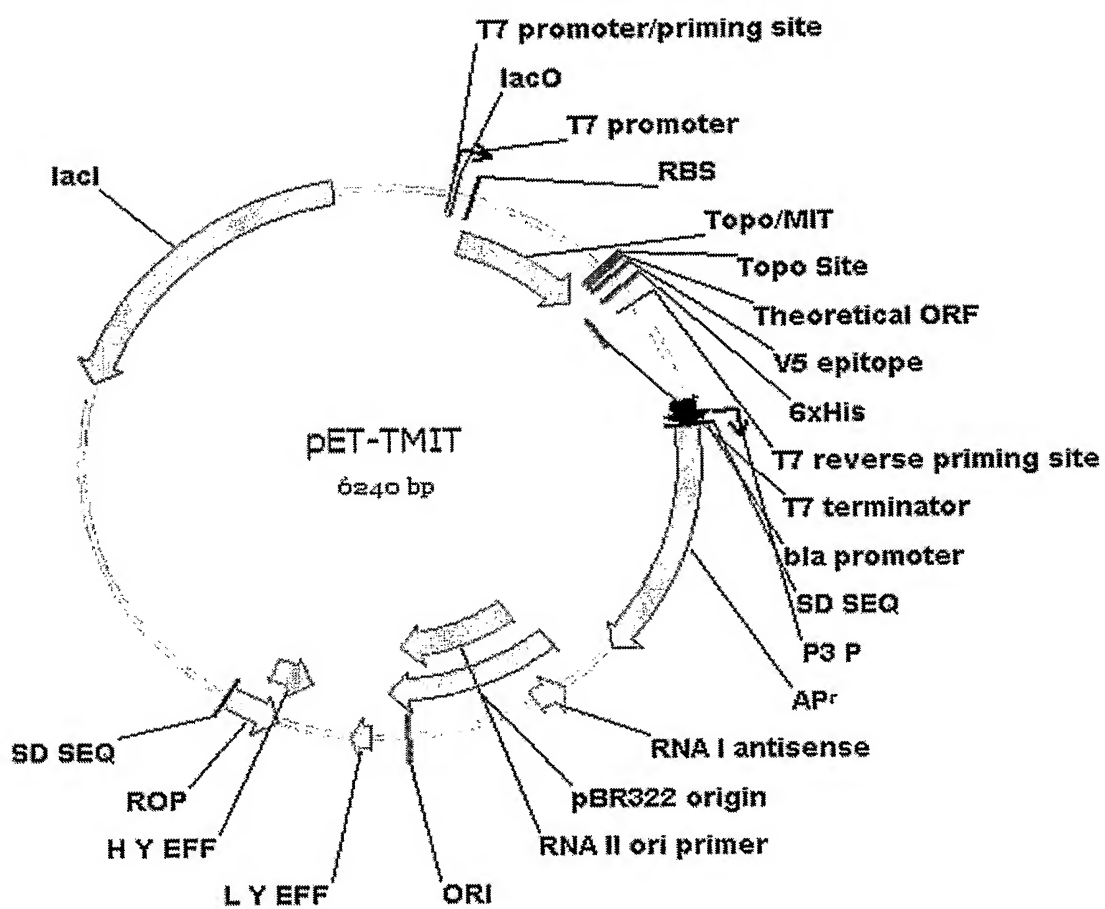


FIG. 7

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## Conserved C-terminus of the Intein

Gene	N-Extein	Intein Sequence			C-Extein
Sce VMA	A I L Y V G	C F A K G T	-( 454 AA )-	N Q V V V H N	C G E R G N
Ctr VMA	V I I Y V G	C F T K G T	-( 471 AA )-	N M A L V H N	C G E R G N
Mtu recA	K V V K N K	C L A E G T	-( 440 AA )-	E G V V V H N	C S P P F K
Mle recA	I G V M F G	C M N Y S T	-( 365 AA )-	D G V M V H N	S P E T T T
Tli pol I2	K V L Y A D	S V S G E S	-( 390 AA )-	N N I L V H N	T D G F Y A
Tli pol I1	I K L L A N	S I L P N E	-( 538 AA )-	G L L Y A H N	S Y Y G Y M
Psp pol	I K I L A N	S I L P E E	-( 537 AA )-	G F L Y A H N	S Y Y G Y Y

**FIG. 8**



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## Topo Intein Designs

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Original Intein Coding Sequence	TS Coding Sequence
<div style="display: flex; align-items: center;"> <div style="flex: 1; border-bottom: 1px solid black; position: relative; margin-bottom: 5px;"> <span style="position: absolute; right: -5px; top: -5px;">→</span> </div> </div>	<div style="display: flex; align-items: center;"> <div style="flex: 1; border-bottom: 1px solid black; position: relative; margin-bottom: 5px;"> <span style="position: absolute; right: -5px; top: -5px;">→</span> </div> </div>
GTT GTT GTA CAC AAC TGT	ATG AAA CAA TAC
CAA CAA CAT GTG TTG ACA	TAC TTT GTT ATG
Val Val Val His Asn Cys	Met Lys Gln Tyr

### "ALV Intein"

Topo Mutations

GCC CTT GTA CAC AAC TGT	ATG AAA CAA TAC
CTT GAA CAT GTG TTG ACA	TAC TTT GTT ATG
Ala Leu Val His Asn Cys	Met Lys Gln Tyr

### "VLV Intein"

Topo Mutations

GTC CTT GTA CAC AAC TGT	ATG AAA CAA TAC
CAT GAA CAT GTG TTG ACA	TAC TTT GTT ATG
Val Leu Val His Asn Cys	Met Lys Gln Tyr

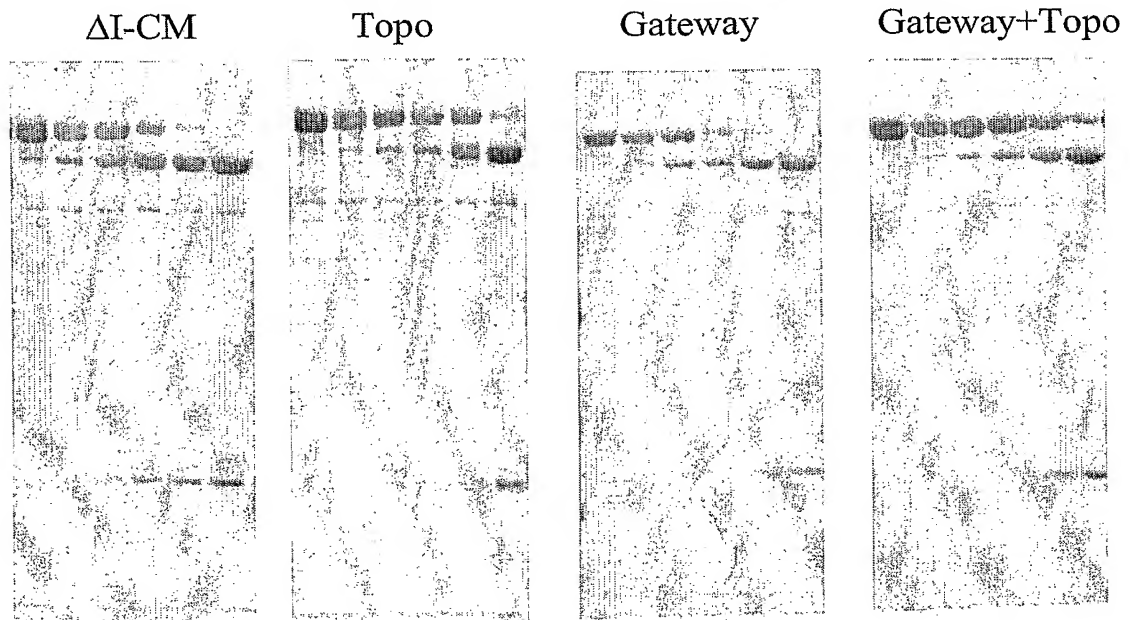
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FIG. 9

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### Cleaving Rate Studies

(Cleaving at pH 7.0 for 0, 1, 2, 4, 8 and 22 hrs)

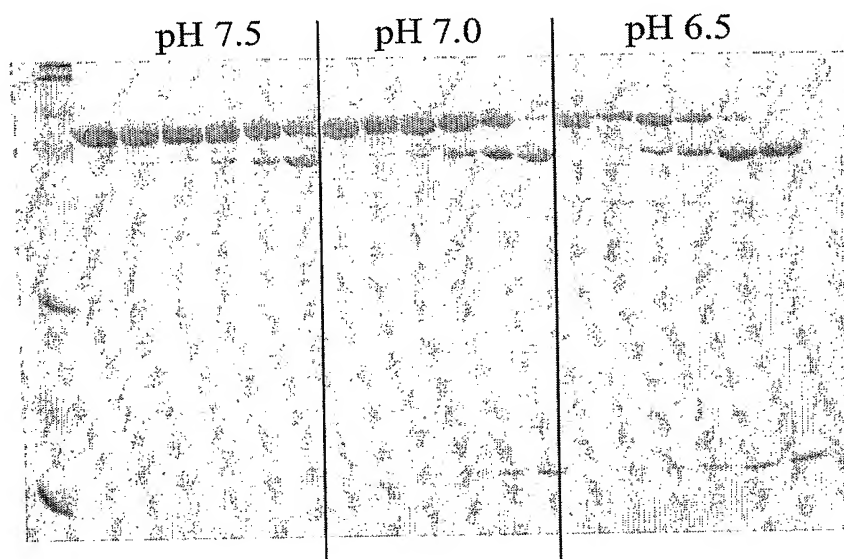


**FIG. 10**

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## **Cleaving Rate Studies - Optimal pH**

(Cleaving at 37°C)



**FIG. 11**